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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
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International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/05910	05.06.2003	10.06.2002

International Patent Classification (IPC) or both national classification and IPC
G01N33/68

Applicant
EVOTEC NEUROSCIENCES GMBH et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
17.12.2003	30.08.2004

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/05910

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-29 as originally filed

Claims, Numbers

1-12 received on 05.07.2004 with letter of 05.07.2004

Drawings, Sheets

1/9-9/9 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 3-4 and 6-8

   because:

   ☒ the said international application, or the said claims Nos. 6-8 for IA relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 3-4 (incomplete)

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/ or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-2 and 10
	No:	Claims	3-9 and 11-12
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12
Industrial applicability (IA)	Yes:	Claims	1-5 and 9-12
	No:	Claims	

2. Citations and explanations

see separate sheet

Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 6-8 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT.
Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

On **claims 3**, referring to reagents selectively detecting a transcription or translation product of the steroidogenic acute regulatory protein (StAR), and **4**, referring to a modulator of G3BP2, only a limited search, restricted to antibodies and antisense oligonucleotides binding to StAR has been performed, and therefore only a limited opinion, restricted to said subject-matter and known modulators of StAR, will be given on said claims (see also 4.2)

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: STOCCO DOUGLAS M: 'Tracking the role of a StAR in the sky of the new millennium.' MOLECULAR ENDOCRINOLOGY, vol. 15, no. 8, August 2001 (2001-08), pages 1245-1254

D2: KALLEN C B ET AL: 'UNVEILING THE MECHANISM OF ACTION AND REGULATION OF THE STEROIDOGENIC ACUTE REGULATORY PROTEIN' MOLECULAR AND CELLULAR ENDOCRINOLOGY, AMSTERDAM, NL, vol. 145, no. 1/2, 25 October 1998 (1998-10-25), pages 39-45

D3: CARON KATHLEEN M ET AL: 'Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipoid adrenal hyperplasia.' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 94, no. 21, 1997, pages 11540-11545

D4: WO 01 32920 A (METRIS THERAPEUTICS LTD ;PAPPA HELEN (GB); LNENICEK MIRNA (GB)) 10 May 2001 (2001-05-10)

D5: WO 00 66728 A (SAVITZKY KINNERET ;COMPUGEN LTD (IL); MINTZ LIAT (IL)) 9 November 2000 (2000-11-09)

D6: KIMOTO TETSUYA ET AL: 'Neurosteroid synthesis by cytochrome P450-containing systems localized in the rat brain hippocampal neurons: N-methyl-D-

aspartate and calcium-dependent synthesis.' ENDOCRINOLOGY, vol. 142, no. 8, August 2001 (2001-08), pages 3578-3589

D7: WO 99 52519 A (GEN HOSPITAL CORP) 21 October 1999 (1999-10-21)

D8: US-A-5 556 847 (JOHNSON DAVID A ET AL) 17 September 1996 (1996-09-17)

The document D9 was not cited in the international search report. A copy of the document is appended hereto:

D9: WO0131342 (TULARIK INC (US)) 3 May 2001 (2001-05-03)

1. Novelty

The subject-matter of **claims 3-9 and 11-12** is not new in the sense of Article 33(2) PCT.

1.1. D9 (p 43, par 1) discloses a kit, which is suitable for diagnosing AD, according to present **claim 3**.

1.2. Numerous modulators of either the StAR gene or its transcription or translation product are known, rendering **claim 4** not novel:
modulators of the StAR gene and its transcription product: D1 (p 1246, co 2, par 2 to p 1247, co 2, par 3) and D9 (p 39, par 1)
modulators of the StAR protein: D2 (p 42, co 1, par 2) and D9 (p 39, par 1)

1.3. D3 discloses a StAR knock-out mouse, D4 (claims 55 and 56) describes genetically-modified non-human animals that have been transformed to express higher, lower or absent levels of e.g. StAR and also D9 describes the manufacture of StAR-transgenic animals. The described StAR transgenic animals are bound to have a predisposition for AD, thereby anticipating **claim 5**.

1.4. D9 (p 35, par 2) discloses a method of screening for agents, suitable for modulating a neurodegenerative disease, comprising the steps a-d of **claim 6**.

1.5. D9 (p 43, par 3) furthermore mentions the use of the transgenic animals for the development of potential treatments according to present **claims 7 and 8**.

1.6 D9 (p 35, par 2 and p 43, par 3) therefore also anticipates present **claim 11**, since it discloses the use of StAR as screening target for developing agents, which are suitable for treating neurodegenerative diseases.

1.7. Moreover, competitive ligand binding assays for screening compounds as described in **claim 9** are known from document D9 (p 24, li 20 till p 26, li 18):
D9 describes a competitive immunoassay using labelled and non-labelled antibodies.

1.8. D9 (p 3, li 11 till p 4, li 4) anticipates moreover the subject-matter of **claim 12** (use of an antibody immunoreactive with the StAR protein for detection of a pathological state of a cell) is (fig. 1). Described is a method of detecting a cancer, involving the histochemical use of antibodies.

The subject-matter of **claims 1-2 and 10**, relating to the use of StAR for diagnosing neurodegenerative diseases, is novel.

2. Inventive Step

The Application furthermore does not comply with Art. 33(3) PCT, because **claims 1-2 and 10** do not comprise an inventive step:

2.1. Document D8 is considered to represent the most relevant state of the art. Document D8 (co 1, li 1 till co 2, li 20) discloses, that decreased levels of pregnenolonesulfate (PREGS) are indicative of cognitive impairments typical for AD. Patients suffering from said disease therefore benefit from the increase of PREGS. From this the subject-matter of **claims 1-2 and 10** differs in that another marker is used for diagnosing neurodegenerative diseases. The problem to be solved by claims 1 and 2 is therefore to provide further diagnostic markers for neurodegenerative diseases. The solution, to analyse the levels and activity of the StAR gene and/ or its products, can however not be considered as containing an inventive step for the following reasons: The person skilled in the art knows, that the StAR protein initiates the synthesis of PREGS (D2, abstract) and is actually the rate-limiting step for the synthesis of PREGS.
The choice of an enzyme as a diagnostic marker for neurodegeneration, which is rate-limiting for production of neuroprotective neurosteroids, therefore does not constitute an inventive step.

2.2. Even if an inventive step for **claims 1-2 and 10** would be acknowledged, then the Applicant is only providing data, demonstrating the diagnostic use of StAR for AD. The Applicant however claims a large number of different neurodegenerative disorders. The prevailing opinion is, that most of said diseases have a different molecular origin, to such an extent, that detection of StAR will not provide a diagnosis for all of them. Since detection of StAR does not provide a solution for all diseases claimed, claims 1 and 10 furthermore lack an inventive step for their incapability to solve the problem posed.

3. Industrial Applicability

Claims 1-5 and 9-12 are industrially applicable.
For the assessment of the present **claims 6-8,** phrased in a way, that they may comprise treatment steps, practiced on the human body, on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. Claim 6 refers to any cell, also a cell, being part of a human being. Claims 7-8 refer to test animals, a term which clearly comprises human beings. Claims 7 and 8 therefore also refer to clinical trials in humans.

4. Clarity and support

The claims do not comply with the requirements of Article 6 and Rule 6.3 (a) PCT for the following reasons:
4.1. The use of the phrases "fragment, derivative or variant" (in **claims 1-2 and 4-12**) leads to a lack of clarity, particularly in view of the definitions found at pages 4 and 6 of the present application, which merely add to the confusion as to the precise scope of the claims.
4.2. The definition of the reagents in **claim 3** and the term "modulator" in **claim 4** is purely functional, based merely on results to be achieved, but completely lacking any technical features.
4.3. The following formulations are relative terms without well-recognized meaning and therefore unclear:

- "increased risk of developing said disease" and "reference value representing a known disease or health status" in claims 1 and 2
- "related diseases" in claim 6
- "pathological state" and "wherein an altered degree of staining or altered staining pattern ... compared to a cell representing a known health status" in claim 12

4.4. **Claims 1-2** furthermore lack support in terms of technical features with regard to exactly how the diagnosis, prognostication and predisposition is actually determined.
4.5. **Claim 3**, directed to a kit for diagnosing a neurodegenerative disease, contradicts Art. 6 PCT, because it mainly relates to a method of using the kit rather than clearly defining the kit in terms of technical features. The intended limitations are therefore not clear from this claim.

5. Disclosure

The Application is furthermore in contradiction to Article 5 PCT for the following reasons:
The Applicant discloses a down-regulation of the StAR gene transcription in the

temporal cortex of Alzheimer`s disease (AD) patients versus healthy controls and versus the frontal cortex of the same patients.
It is neither explained in the description nor clear to the person skilled in the art, how to use this finding in a method of diagnosis according to **claims 1 and 2**, for the following reasons: It is not clear, which degree of expression is considered normal and which as indicative of a neurodegenerative process. On the basis of figure 3, depicting the amplification kinetics of RT-PCR products collected from different brain regions of an AD patient and a healthy control, the Applicant claims, that a significant difference between affected and unaffected brain exists, without explaining however, what the term "significant" means. The graph depicted in fig. 3, lacks moreover a scaling of the axes, making it impossible for the skilled person, to follow the Applicants reasoning.
The Applicant does not disclose any data, indicating, that StAR is involved in the pathogenesis of neurodegenerative diseases, rendering **claims 6-8 and 11**, which refer to identification of modulators of neurodegenerative diseases by using StAR as a screening target, mere speculation.

AMENDED CLAIMS

1. A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising determining a level and/or an activity of

(i) a transcription product of the gene coding for the steroidogenic acute regulatory protein, and/or

(ii) a translation product of the gene coding for the steroidogenic acute regulatory protein , and/or

(iii) a fragment, or derivative, or variant of said transcription or translation product,

in a sample obtained from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

2. The method according to claim 1 wherein said neurodegenerative disease is Alzheimer's disease.

3. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease by:

(i) detecting in a sample obtained from said subject a varied, or a similar or equal level, or activity, or both said level and said activity of a transcription product and/or of a translation product of a gene coding for the steroidogenic acute regulatory protein compared to a reference value representing a known health status, or representing a known disease status;

and said kit comprising:

a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of a gene coding for the steroidogenic acute regulatory protein, and (ii) reagents that selectively detect a translation product of a gene coding for the steroidogenic acute regulatory protein.

4. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of

(i) the gene coding for the steroidogenic acute regulatory protein, and/or

(ii) a transcription product of the gene coding for the steroidogenic acute regulatory protein, and/or

(iii) a translation product of the gene coding for the steroidogenic acute regulatory protein, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii).

5. A recombinant, non-human animal comprising a non-native gene sequence coding for the steroidogenic acute regulatory protein, or a fragment, or a derivative, or a variant thereof, said animal being obtainable by:

(i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and

(ii) introducing said targeting construct into a stem cell of a non-human animal, and

(iii) introducing said non-human animal stem cell into a non-human embryo, and

(iv) transplanting said embryo into a pseudopregnant non-human animal, and

(v) allowing said embryo to develop to term, and

(vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and

(vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease or related diseases or disorders.

6. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) the gene coding for the steroidogenic acute regulatory protein, and/or

(ii) a transcription product of the gene coding for the steroidogenic acute regulatory protein, and/or

(iii) a translation product of the gene coding for the steroidogenic acute regulatory protein, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

(a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

(d) comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

7. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) the gene coding for the steroidogenic acute regulatory protein, and/or

(ii) a transcription product of the gene coding for the steroidogenic acute regulatory protein, and/or

(iii) a translation product of the gene coding for the steroidogenic acute regulatory protein, and/or

(v) a fragment, or derivative, or variant of (i) to (iii),

said method comprising:

(a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect of the substances recited in (i) to (iv);

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) or (iv) in a matched control animal which is predisposed to developing or has already developed a neurodegenerative disease or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which animal no such test compound has been administered;

(d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases or disorders.

8. The method according to claim 7 wherein said test animal and/or said control animal is a recombinant animal which expresses the

steroidogenic acute regulatory protein, or a fragment, or a derivative, or a variant thereof, under the control of a transcriptional control element which is not the native steroidogenic acute regulatory protein gene transcriptional control element.

9. An assay for testing a compound, preferably for screening a plurality of compounds for inhibition of binding between a ligand and a translation product of the gene coding for the steroidogenic acute regulatory protein, or a fragment, or a derivative, or a variant thereof, said assay comprising the steps of:

(i) adding a liquid suspension of said translation product of the gene coding for the steroidogenic acute regulatory protein, or a fragment, or derivative, or variant thereof, to a plurality of containers;

(ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers;

(iii) adding a detectable, preferably a fluorescently labelled ligand to said containers;

(iv) incubating said translation product of the gene coding for the steroidogenic acute regulatory protein, or said fragment, or derivative, or variant thereof, and said compound or compounds, and said detectable, preferably fluorescently labelled ligand;

(v) measuring amounts of preferably fluorescence associated with said translation product of the gene coding for the steroidogenic acute regulatory protein, or with said fragment, or derivative, or variant thereof; and

(vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said translation product of the gene coding for the steroidogenic acute regulatory protein, or said fragment, or derivative, or variant thereof.

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10. Use of a protein molecule, said protein molecule being a translation product of the gene coding for the steroidogenic acute regulatory protein, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

11. Use of a protein molecule, said protein molecule being a translation product of the gene coding for the steroidogenic acute regulatory protein, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

12. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the gene coding for the steroidogenic acute regulatory protein, SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for detecting a pathological state of a cell in a sample obtained from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell.